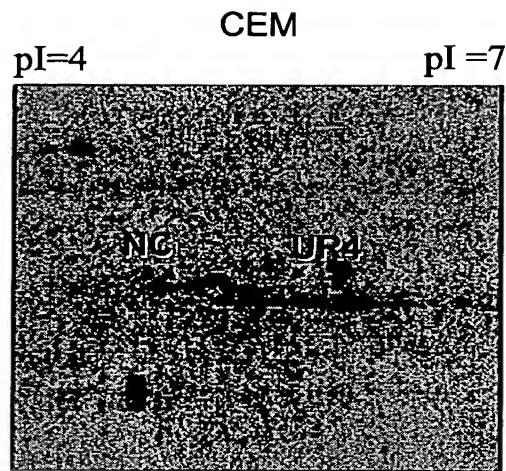
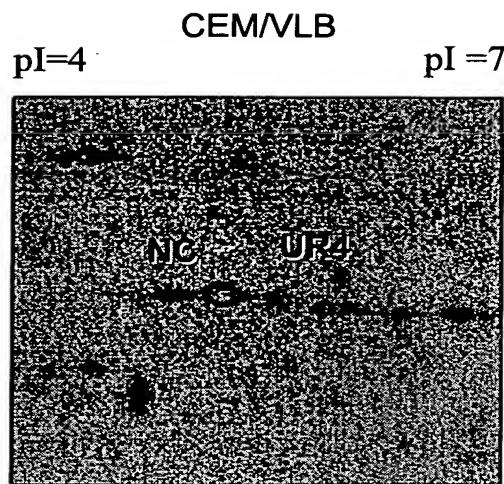




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**FIG. 1A**



**FIG. 1B**

## Isoform 2 Results

ProFound -Search Result Summary			
Protein Candidates for search 20010608200436-0394208172123151 [73182 sequences searched]			
Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)
+1	1.0e+000	2.36	gi 5729877 ref NP_006588.1 Heat shock 70 kD protein 8, heat shock cognate protein, 71 kDa,heat shock 70kd protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens]

FIG. 2A

## Search Parameters

Details for rank 1 candidate in search 20010608200436-0394208172123151
gi 5729877 ref NP_006588.1 heat shock 70 kD protein 8, heat shock 70kD protein 8 (HSP73), heat shock cognate protein, 71 kDa,heat shock 70kd protein 10(HSC71) [Homo sapiens]
gi 13639862 ref XP_006086.2  heat shock 70kD protein 8 [Homo sapiens]
gi 123648 sp P11142 HS7C HUMAN HEAT SHOCK COGNATE 71 KDA PROTEIN
gi 87625 pir  IA27077 dnaK-type molecular chaperone-human
gi 32467 emb CAA68445.1 (Y00371)71 Kd heat shock cognate protein [Homo sapiens]
gi 13273304 gb AAK17898.1 AF352832_1(AF352832) constitutive heat shock protein 70 [Homo sapiens]
Sample ID NO CHANGE [Pass 0]
Measured peptides 20
Matched peptides 12
Min. sequence coverage 25%

FIG. 2B

<u>12 Peptides Sequenced*</u>	Measured Mass (M)	Avg/ Computed Mono Mass	Error (ppm)	Start Residues	To Cut	Peptide sequence	Missed
-1	1250.623	M 1250.611	10	237	246	0 MVNHFIAEFK	(1) +O@M;
-2	1252.592	M 1252.608	-13	302	311	0 FEELNADLFR	
-3	1406.696	M 1406.712	-12	237	247	1 MVNHFIAEFKR	(1) +O@M;
-4	1479.743	M 1479.746	-2	300	311	1 ARFEELNADLFR	
-5	1486.756	M 1486.693	42	37	49	0 TTPSYVAFTDTER	
-6	1690.705	M 1690.718	-7	221	236	0 STAGDTHLGGEDFDNR	
-7	1786.968	M 1786.982	-8	172	188	1 IINEPTAAIAYGLDKK	
-8	1820.874	M 1820.883	-5	57	72	1 NQVAMNPNTVFDAKR	(1) +O@M;
-9	1837.001	M 1837.005	-2	326	342	1 LDKSQIHIDIVLVGGSTR	
-10	1951.065	M 1951.052	7	452	469	1 DNNLLGKFELTGIPPAPR	
-11	1980.996	M 1980.990	3	138	155	0 TVTNAVVTVPAYFNDNSQR	
-12	2773.258	M 2773.318	-21	424	447	0 QTQTFTTYSDNQQPGVLIQVYEGER	

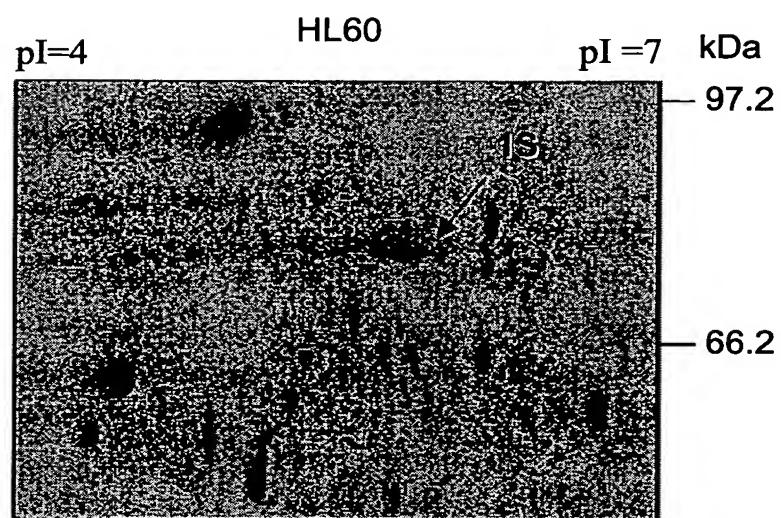
\*25% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed.

FIG. 2C

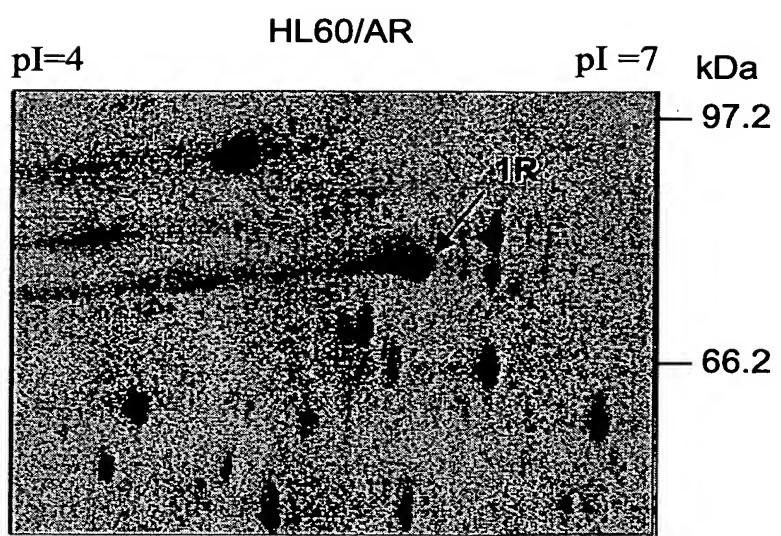
1 MSKGPAVGIDLGTTYSCTVGVFQHGIKVEIIANDQGINRTTPSYVAFTDTERLIGDAAKNQVA  
 61 MNPTNTVFDAKRLLIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGGETKSFYPEEV  
 121 SMVLTKMKIEAAYLGKTVTNAVVTVPAYFINDSQRQATKDAAGTIAGLNVLRIINEPTAA  
 181 IAYGLDKKKVGAERNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNH  
 241 FIAEFKRKHKKDISENKRAWRRLRTACERA  
 KRTLSSSTQASIEIDSLYEGIDFYTSITRA  
 301 RFEELNADLFRGTLDPVEKA  
 RLDAKLDKSQIHDIVLVGGSTRIPKIQKLQLQDFNGKELN  
 361 KSINPDEAWAYGAAVQAAILSGDKSENVQDLLLDVTPLSLGIETAGGYUMTVLIKRNNTI  
 421 PTKQTQTFTTYSDNQPGVLIQVYEGERAMTKDN  
 NLLGKFELTGIPPAPRGVPRQIEVTFDI  
 481 DANGILNSAVDKSTGKENKITITNDKGRLSKEDIERMVQEAEKYKA  
 EDEKQRDKVSSKN  
 541 SLESYAFNMKATVEDEKLQGKINDEDKQKILDKCNEII  
 NWLDKNQTAEEKEEFHQKELE  
 601 KVCNPIITKLYQSAGGMPGGMPGGAPPSSGGASSGPTIEVD

**BOLD** = Peptides covered by MS MALDI analysis

FIG. 3



**FIG. 4A**



**FIG. 4B**

ProFound -Search Result Summary				Version 4.10.5 The Rockefeller University Edition			
Protein Candidates for search BD36B5S9-CG5C-289FB336 [88967 sequences searched]							
Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	pI	kDa	(R)
+1	1.0e+000	2.43	T gi 5729877 refNP_006588.1  (NM_006597) heat shock 70kd protein 8, heat shock 70kD protein 8(HSP73), heat shock cognate protein 71-kDa, heat shock 70kd protein 10 (HSC71) [Homo sapiens]	<u>26</u>	5.4	71.11	(R)

FIG. 5A

### Search Parameters

Input Summary	Date & Time Mon Dec 17 01 01:33 2001 UTC (Search Time: 2 80 sec)
<b>Sample ID</b>	
<b>Database</b>	NCBInr (2001/12/11)
<b>Taxonomy Category</b>	Homo sapiens (human)
<b>Protein Mass Range</b>	60 - 90 kDa
<b>Protein pI range</b>	4.0 - 5.5
<b>Search for</b>	Single protein only
<b>Digest Chemistry</b>	Trypsin
<b>Max Missed Cut</b>	2
<b>Modifications</b>	+C2H3ON@C(Complete), +O@M(Partial), &nbspC22H37N4O4S@K(Partial);
<b>Charge State</b>	MH+
<b>Peptide Masses</b> (Da,Average)	
<b>Tolerance(AVG)</b>	100.00 ppm
<b>Peptide Masses</b> (Da,Monoisotopic)	1199.667 1253.610 1401.805 1407.785 1463.128 1480.751 1487.698 1691.729 1830.802 1838.043 1966.104 1982.001 1994.000 2231.141 2275.180 2278.081 2757.251 2774.246 2998.417 3548.224
<b>Tolerance(MON)</b>	100.00 ppm
<b>Number of Peptides</b>	20

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FIG. 5B

12 Peptides  
Sequenced\*

Measured peptides	:	20
Matched peptides	:	12
Min. sequence coverage	:	26%

Boxed peptides are biotinylated

-1	1198.659	M	1198.666	-6	160	171	0	DAGTIAGLNVLR					
-2	1252.602	M	1252.608	-5	302	311	0	FEELNADLIFR					
-3	1406.777	M	1406.712	46	237	247	1	MVNRFIAEFKR					
-4	1479.744	M	1479.746	-2	300	311	1	ARFEEINADLIFR					
-5	1486.690	M	1486.693	-2	37	49	0	TPPSYVAFTDTER					
-6	1690.721	M	1690.726	2	221	236	0	STAGDTTHLGGEFDNIR					
-7	1837.035	M	1837.005	16	326	342	1	LDKSQTHDIVLVGGSTR					
-8	1965.096	M	1965.083	7	312	325	2	GTLDPVEKALRDAK					
-9	1980.994	M	1980.990	2	138	155	0	TVTNAVWTVPAYFNDNSOR					
-10	1980.994	M	1980.956	19	518	533	3	MVQFAEKYKADEDEKQR					
-11	2274.272	M	2274.136	16	57	72	1	NQVAAMNPNTNTVFDAKR					
-12	2773.238	M	2773.318	-29	424	447	0	QTQTFTTYSDNQPGVLIQWYEGER					
-13	2997.409	M	2997.452	-14	540	557	1	NSLESYAFNMKATVEDEK					

\*26% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed. Note that 2 and 4 represent the same peptide

FIG. 5C

1 MSKGP AVGIDL GTTYS CVG VFO HQG KVE II AND QGNRTT P S YV AFT DTER LIGDA A K N Q V A  
 61 **MNPTNTVFD** K A R L I G R R F D D A V V Q S D M K H W P F M V V N D A G R P K V Q V E Y K G E T K S F Y P E E V S  
 121 S M V L T K M K E I A E A Y L G K T V T N A V V T V P A Y F N D S Q R Q A T K D A G T I A G L N V L R I I N E P T A A A  
 181 I A Y G L D K K V G A E R N V L I F D L G G G T F D V S I L T I E D G I F E V K S T A G D T H L G G E D F D N R M V N H  
 241 F I A E F K R K H K K D I S E N K R A V R R I L T A C E R A K R T I L S S T O A S I E I D S L Y E G I D F Y T S I T R A  
 301 R F E E L N A D L F R G T L D P V E K A L R D A K L D K S Q I H D I V L V G G S T R I P K I Q K L L Q D F F N G K E L N  
 361 K S I N P D E A V A Y G A A V Q A A I L S G D K S E N V Q D L L L D V T P L S L G I E T A G G V M T V L I K R N T T I  
 421 P T K Q T Q I F T T Y S D N Q P G V L I Q V Y E G E R A M T K D N N N L L G K F F E L T G I P P A P R G V P Q I E V T F D I  
 481 D A N G I L N V S A V D K S T G K E N K I T T N D K G R L S K E D I E R M V Q E A E K Y K A E D E K Q R D K V S S K N  
 541 S L E S Y A F N M K A T V E D E K L Q G K I N D E D K Q K I L D K C N E I I N W L D K N O T A E K E E F E H Q Q K E L E  
 601 K V C N P I I T K L Y Q S A G G M P G G M P G G F P G G A P P S G G A S S G P T I E V D

**BOLD** = Nonbiotinylated peptides specific for HSC70  
*Underlined & Italics* = Biotinylated peptides

FIG. 6

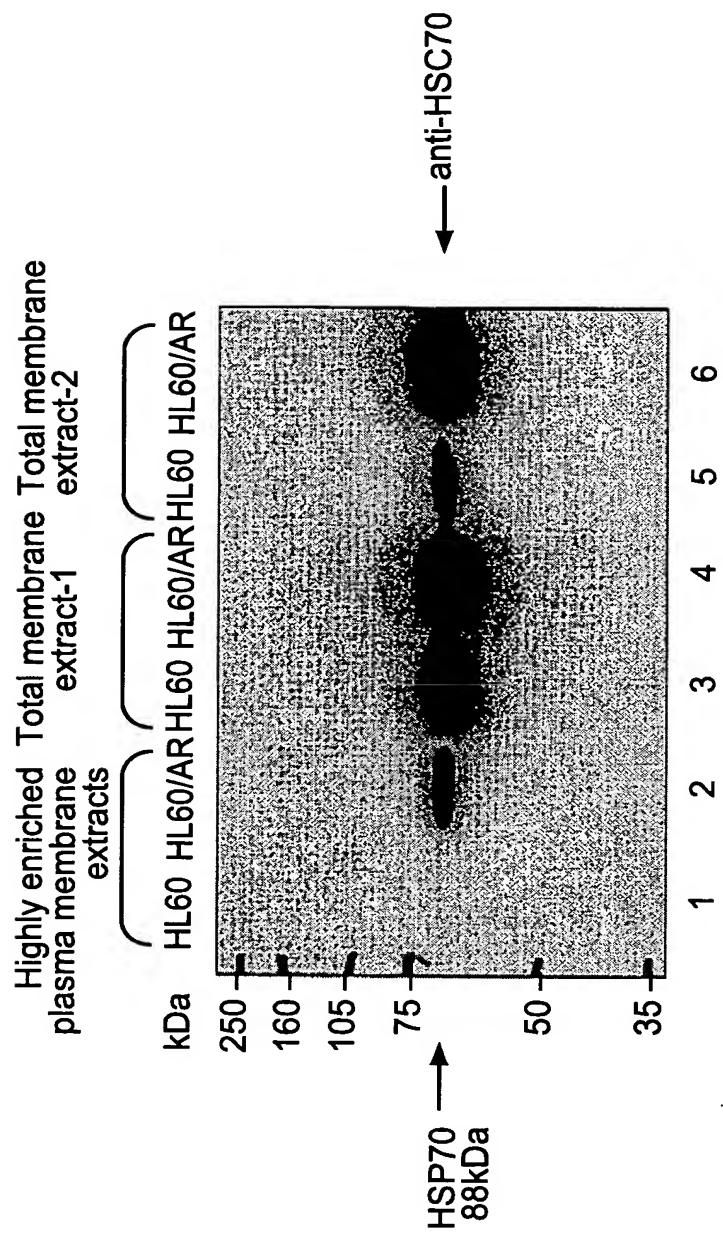
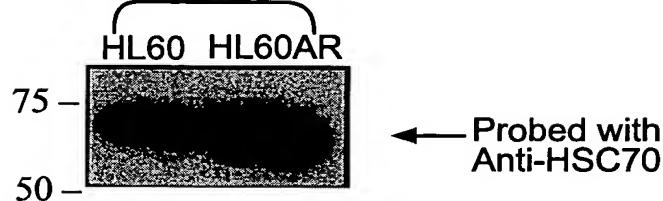


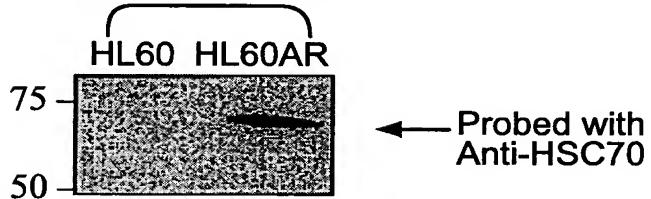
FIG. 7

Biotinylated total cell extracts



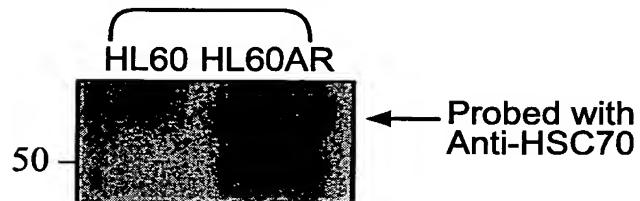
**FIG. 8A**

Streptavidin purified extracts



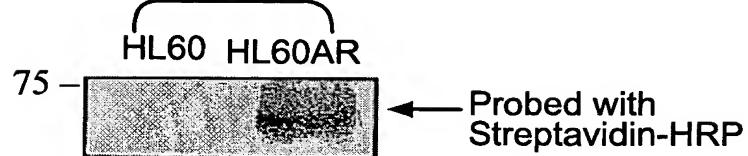
**FIG. 8B**

IP with anti-HSC70



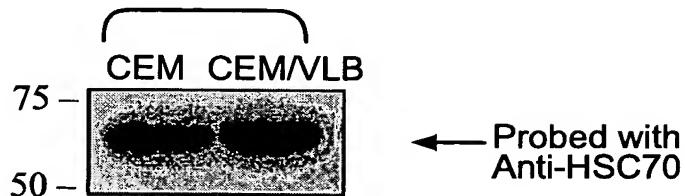
**FIG. 8C**

IP with anti-HSC70



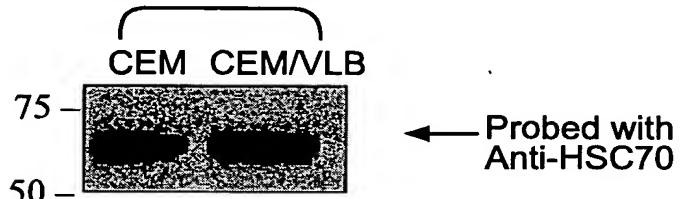
**FIG. 8D**

Biotinylated total cell extracts



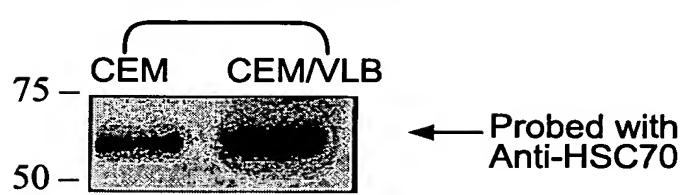
**FIG. 9A**

Streptavidin purified extracts



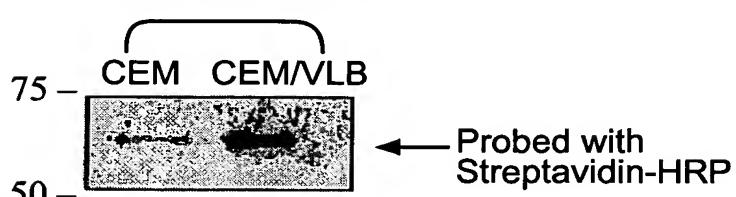
**FIG. 9B**

IP with anti-HSC70



**FIG. 9C**

IP with anti-HSC70



**FIG. 9D**

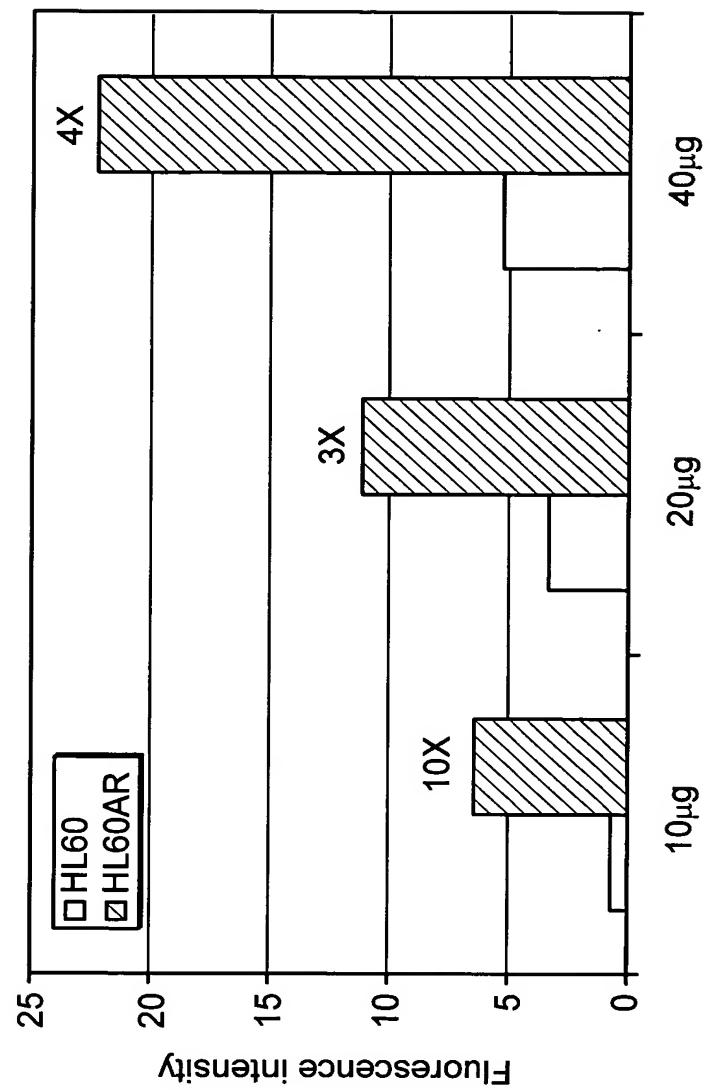


FIG. 10A

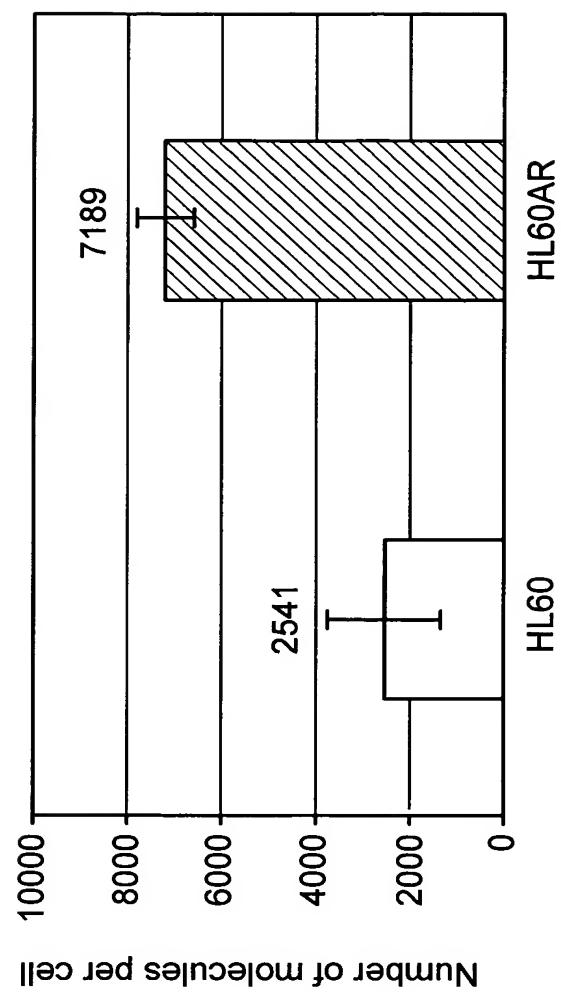


FIG. 10B

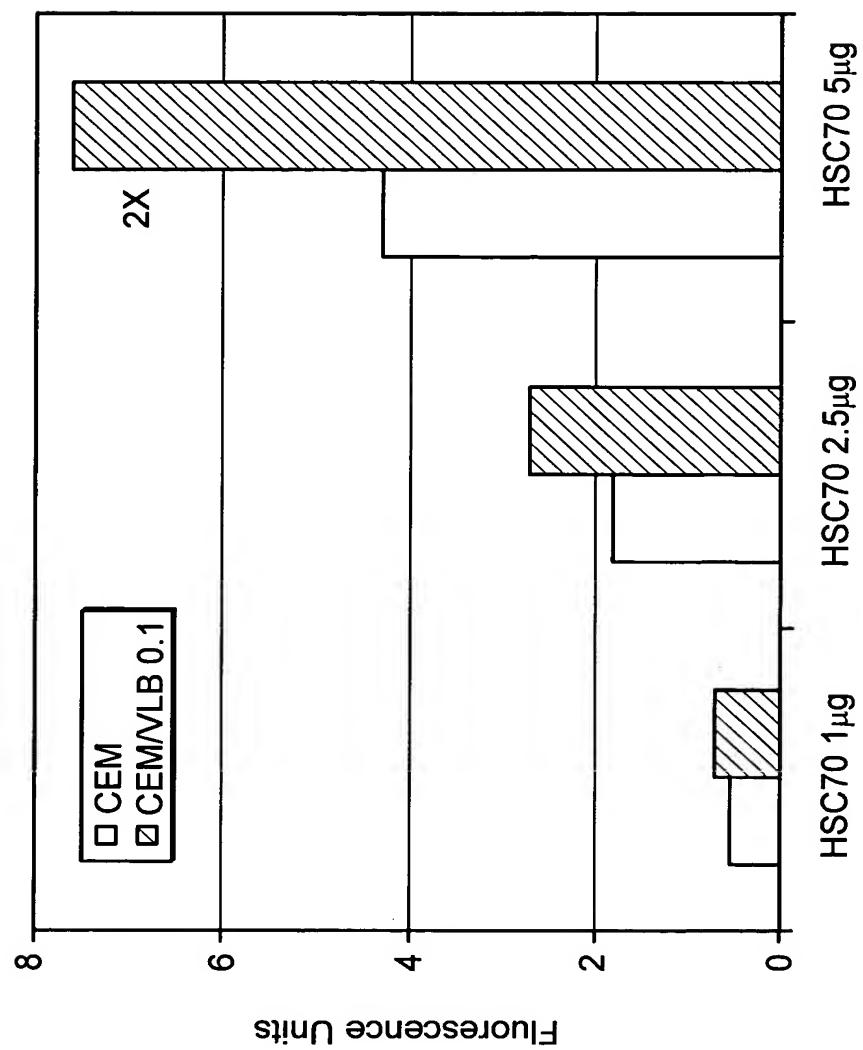


FIG. 11A

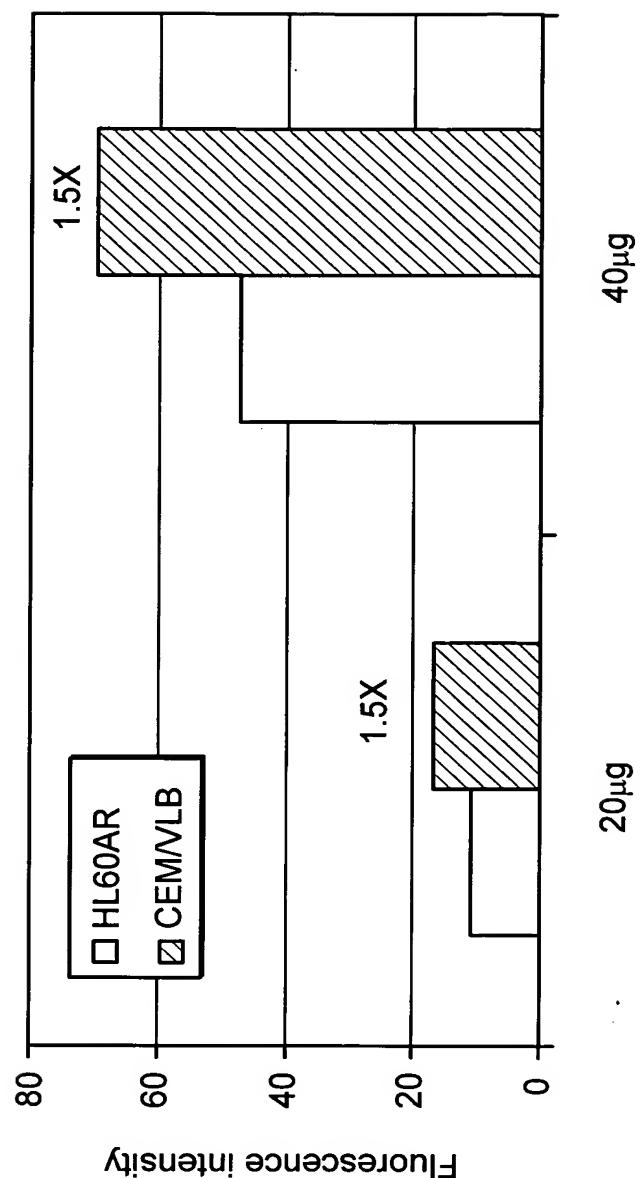


FIG. 11B

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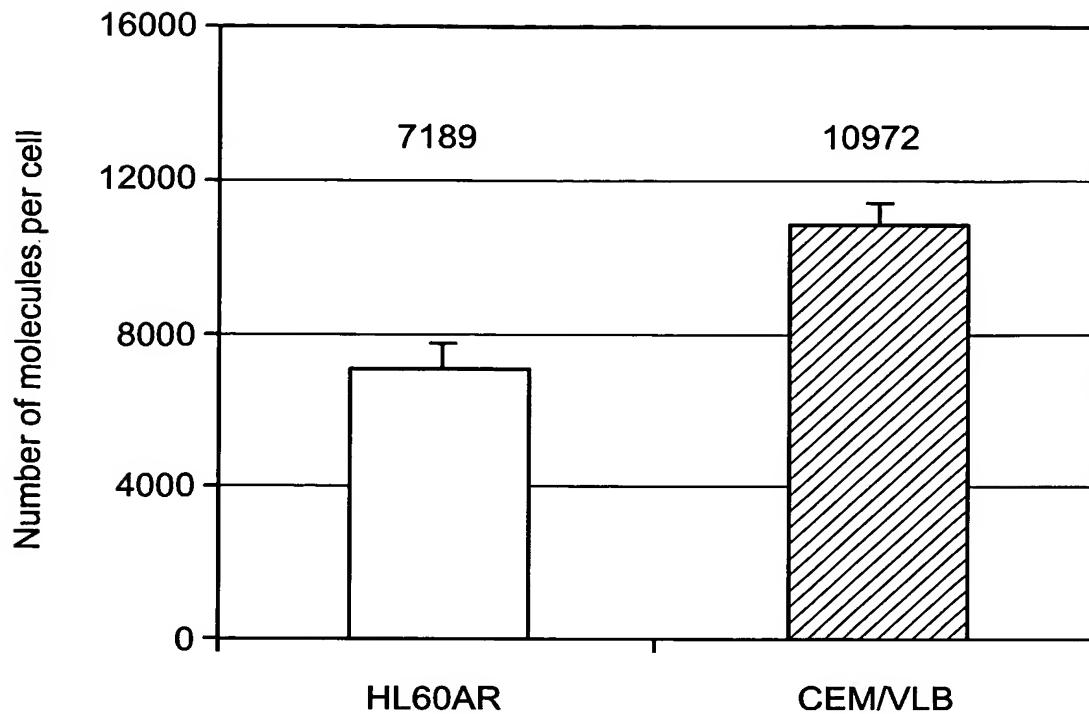


FIG. 11C

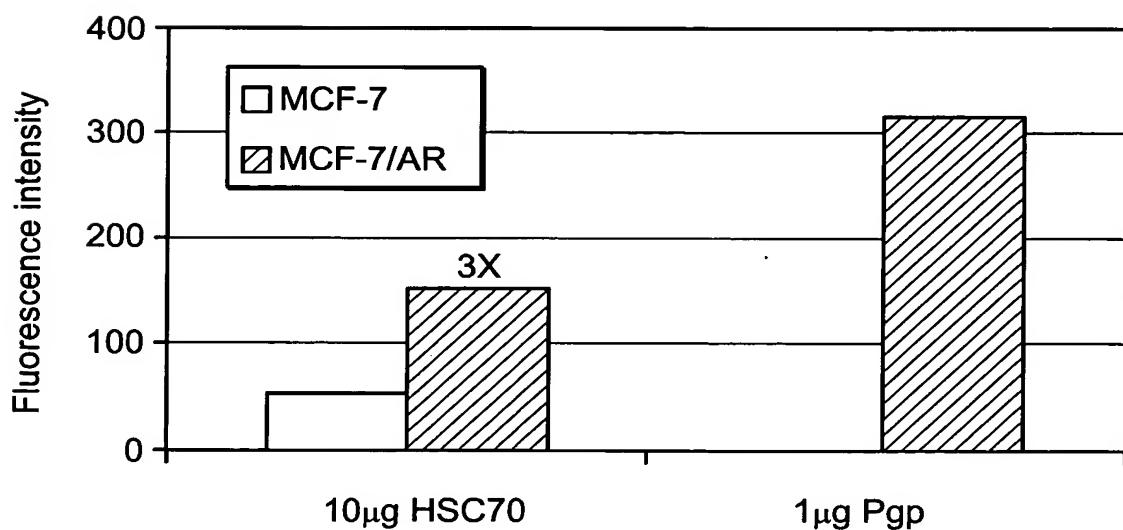


FIG. 12A

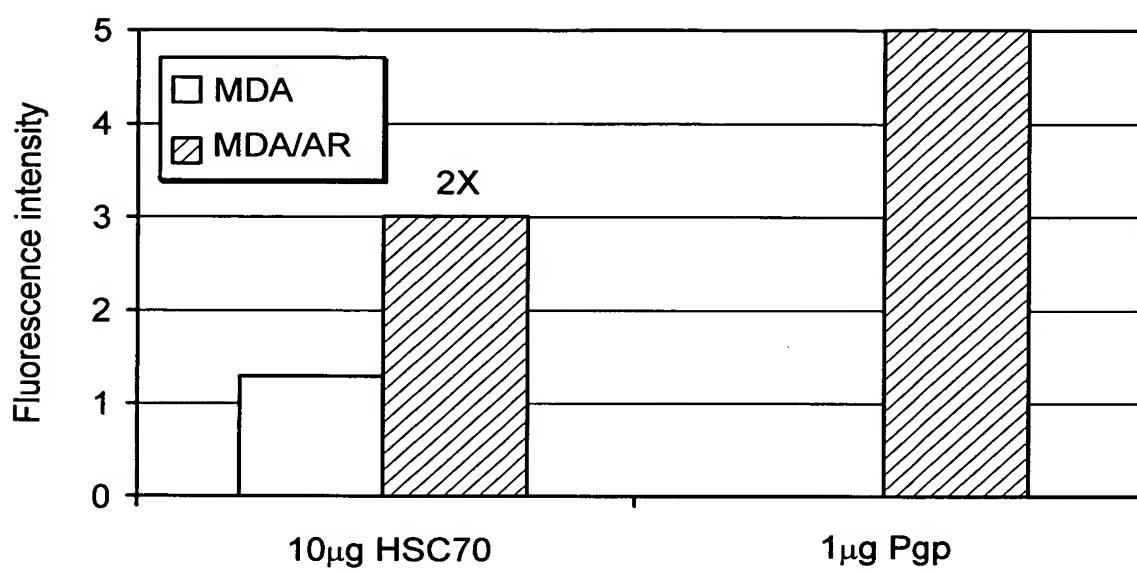


FIG. 12B

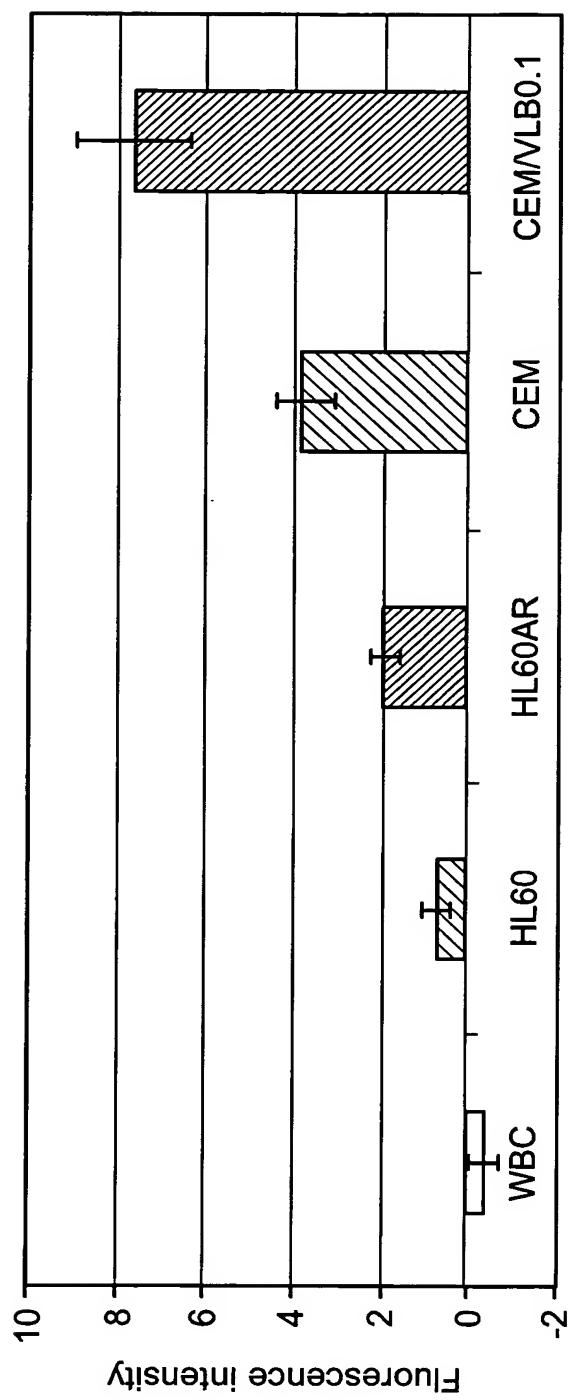


FIG. 13A

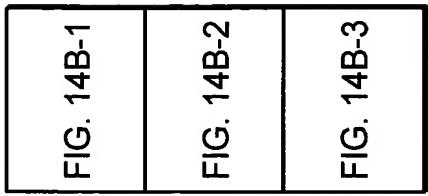
POLYPEPTIDE SEQUENCE OF HUMAN HSC70  
(GENBANK ACCESSION NO. AAK17898 (SEQ ID NO. 1))

1 MSKGPAVGID LGTTYSCVGV FQHKGKVELIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA  
61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR PKVQVEYKGE TKSFYPEEV  
121 SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAAA  
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVN  
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA  
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL QDFFFNGKELN  
361 KSINPDEAVVA YGAAVQAAIL SGDKSENVQD LLLLDVDTPLS LGIETAGGVM TVLIKRN  
421 PTKQTQTFTT YSDNQPGVLI QVYEGERAMT KDNNLLGKFE LTGIPPPAPRG VPQIEVTFDI  
481 DANGILNVSA VDKSTGKENK ITITNDKGRL SKEDIERMVQ EAEKYKAED EKORDKVSSKN  
541 SLESYAFNMK ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAKE EEEHQQKELE  
601 KVCNPPIITKL YQSAGGMMPGG MPGGEPPGGGA PPSGGASSGP TIEEVD

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FIG. 14A

FIG. 14B



DNA SEQUENCE OF HUMAN HUMAN HSC70  
(GENBANK ACCESSION NO. AF352832 (SEQ ID NO. 2))

1 ATGTCCAAGG GACCTGCAGT TGGTATTGAT CTTGGCACCA CCTACTCTTG TGTGGGTGTT  
61 TTCCAGCAG GAAAAGTCGA GATAATTGCC AATGATCAGG GAAACCGAAC CACTCCAAGC  
121 TATGTGGCCT TTACGGACAC TGAAACGGTGTG ATCGGGTATG CCGCAAAGAA TCAAGTTGCA

FIG. 14B-1

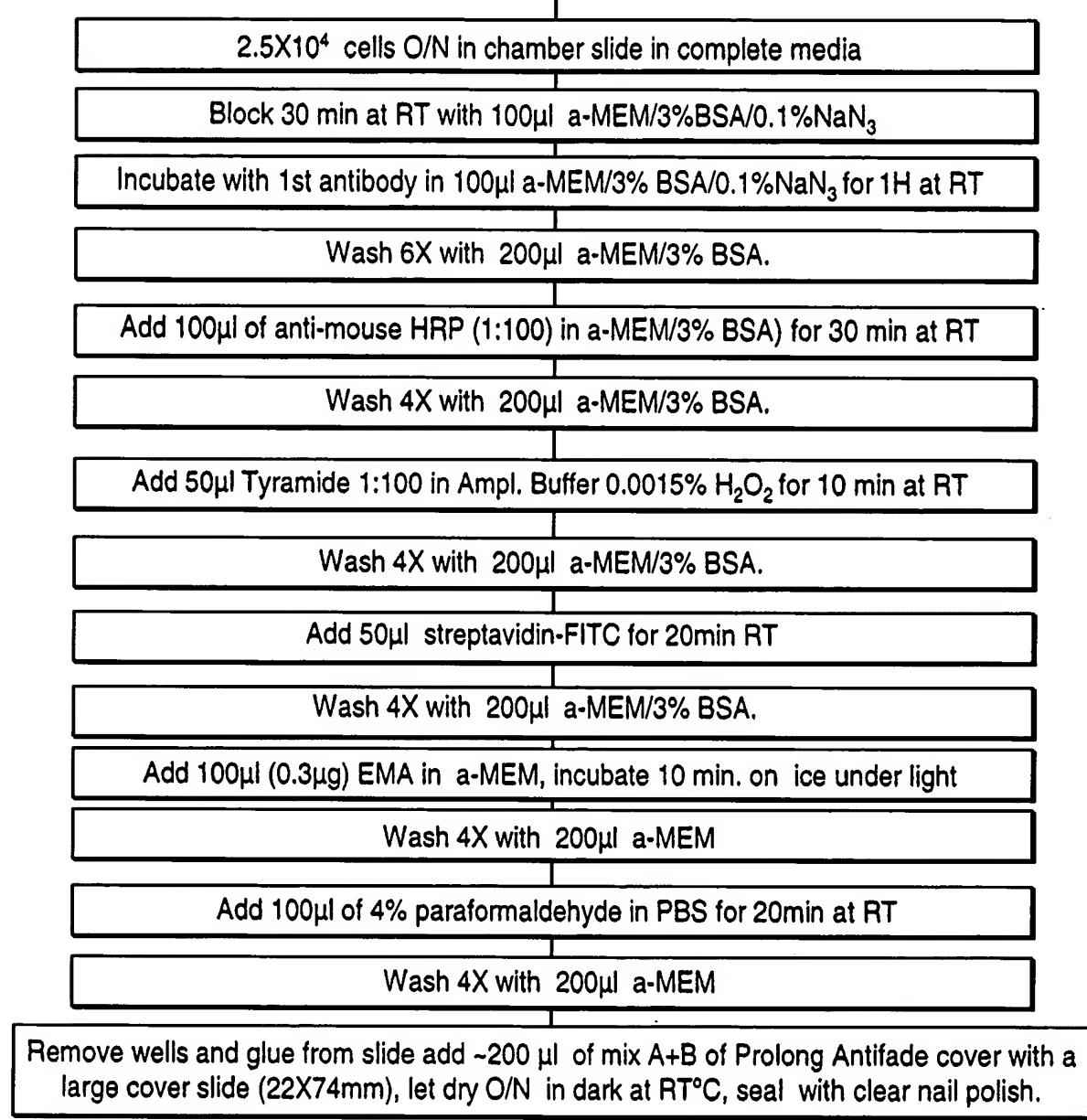
181 ATGAAACCCCA CCAACACAGT TTTGATGCC AACCGTCTGA TTGGACGCAG ATTGATGAT  
241 GCTGTTGTC AGTCTGATAT GAAACATTGG CCCTTTATGG TGGTGAATGA TGCTGGCAGG  
301 CCCAAGGTCC AAGTAGAATA CAAGGGAGAG ACCAAAAGCT TCTATCCAGA GGAGGGTGTCT  
361 TCTATGGTC TGACAAAGAT GAAGGAATT GCAGAACGCCT ACCTTGGAA GACTGTTACC  
421 AATGCTGTC TCACAGTGCC AGCTTACTTT AATGACTCTC AGCGTCAGGC TACCAAAGAT  
481 GCTGGAACTA TTGCTGGTCT CAATGTAATT AGAATTATTAA ATGAGCCAAC TGCTGCTGCT  
541 ATTGCTTACG GCTTAGACAA AAAGGTTGGA GCAGAAAGAA ACGTGCTCAT CTTGACCTG  
601 GGAGGGTGGCA CTTTIGATGT GTCAAATCCTC ACTATTGAGG ATGGATCTT TGAGGTCAAG  
661 TCTACAGCTG GAGACACCCA CTTGGGTGGA GAAAGATTTG ACAAACGAAAT GGTCAACCAT  
721 TTATTGCTG AGTTAAGGG CAAGCATAAG AAGGACATCA GTGAGAACAA GAGAGCTGTA  
781 AGACGCCCTCC GTACTGCTTG TGAACCGTGT AAGCGTACCC TCTCTTCCAG CACCCAGGCC  
841 AGTATTGAGA TCGATTCTCT CTATGAAGGA ATCGACTTCT ATACCTCCAT TACCCGTGCC  
901 CGATTGAG AACTGAATGC TGACCTGTC CGTGGCACCC TGGACCCAGT AGAGAAAGCC  
961 CTTCGAGATG CCAAACCTAGA CAAGTCACAG ATTCAATGATA TTGTCCTGGT TGGTGGTCT  
1021 ACTCGTATCC CCAAGATTCA GAAGCTTCTC CAAGACTTCT TCAATGGAA AGAACTGAAT

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FIG. 14B-2

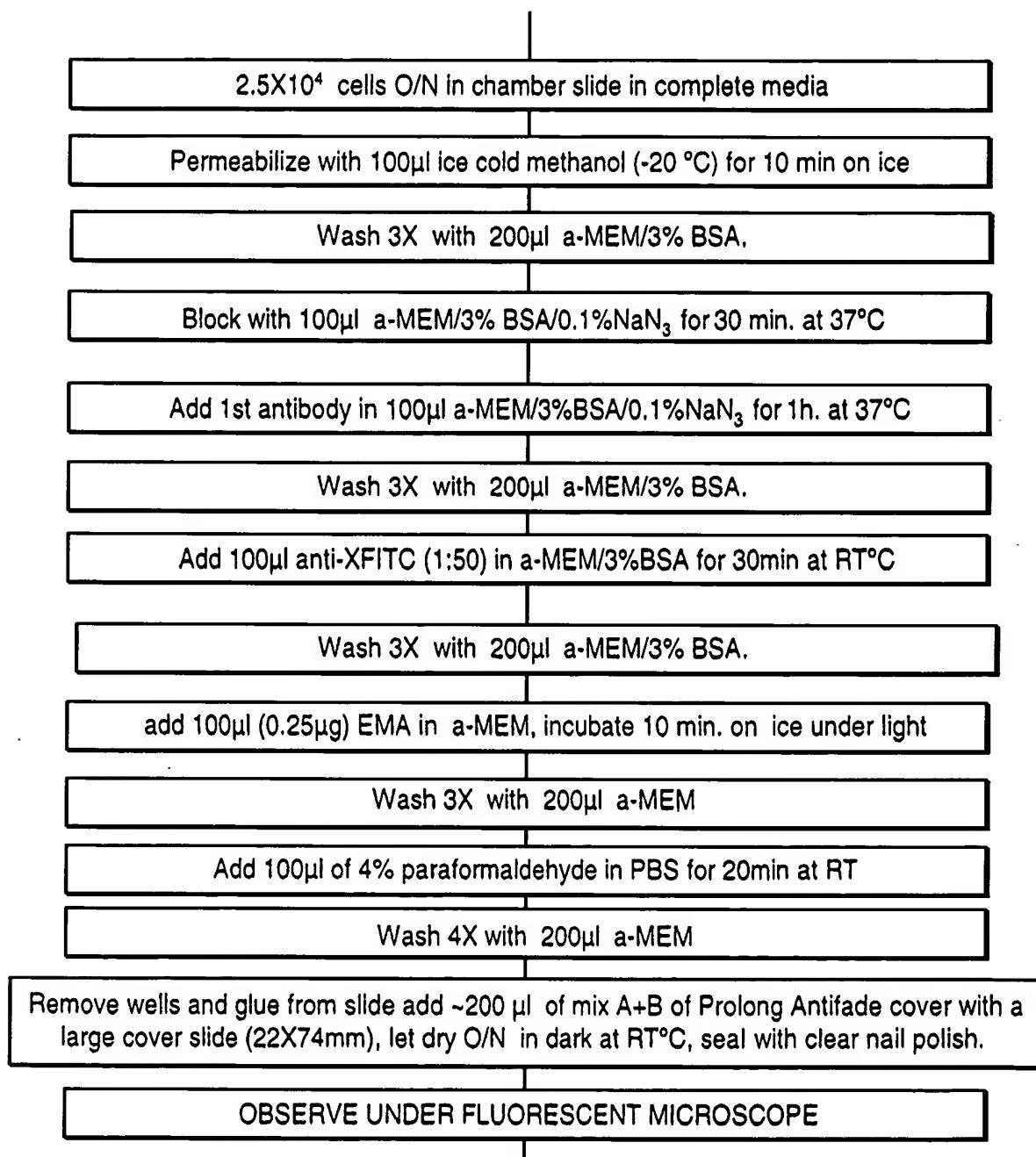
1081 AAGAGGCATCA ACCCCTGATGA AGCTGTGCT TATGGTGCAG CTGTCAGGC AGCCATCTTG  
 1141 TCTGGAGACA AGTCTGAGAA TGTTCAAGAT TTGCTGCTCT TGGATGTCAC TCCTCTTTCC  
 1201 CTTGGTATTG AAACTGCTGG TCGAGTCATG ACTGTCCCTCA TCAAGCGTAA TACCAACCATT  
 1261 CCTACCAAGC AGACACAGAC CTTCACTACC TATTCTGACA ACCAGCCTGG TGTGCTTATT  
 1321 CAGGTTATG AAGGGAGGC TGCCATGACA AAGGATAACA ACCTGGTTGG CAAGTTTGAA  
 1381 CTCACAGGCA TACCTCTGC ACCCGAGGT GTTCCTCAGA TTGAAGTCAC TTTGACAT  
 1441 GATGCCAATG GTATACTCAA GTGGACAAGA GTACGGAAA AGAGAACAAAG  
 1501 ATTACTATCA CTAATGACAA GGGCCGTTG AGCAAGGAAG ACATTGAAACG TATGGTCCAG  
 1561 GAAGCTGAGA AGTACAAAGC TGAAGATGAG AACAGAGGG ACAAGGTGTC ATCCAAGAAAT  
 1621 TCACTTGAGT CCTATGCCCT CAACATGAAA GCAACTGTTG AAGATGAGAA ACTTCAAGGC  
 1681 AAGATTAACG ATGAGGACAA ACAGAAAGAT CTGGACAAGT GTAATGAAAT TATAAACTGG  
 1741 CTTGATAAGA ATCAGACTGC TGAGAAGGAA GAATTGAAAC ATCAACAGAA AGAGCTGGAG  
 1801 AAAGTTGCA ACCCCATCAT CACCAAGCTG TACCAAGCTG CAGGAGGCAT GCCAGGGAGGA  
 1861 ATGCCTGGGG GATTTCCTGG TGTTGGAGCT CCTCCCTCTG GTGGTGTCTC CTCAGGGCCC  
 1921 ACCATTGAAG AGGTTGATTA

FIG. 14B-3

**Procedure for immunofluorescence (non-permeabilized cells)**

Amplification kit used:  
 TSA kit #2 with  
 HRP-goat anti-mouse  
 IgG and Alexa fluor 488  
 tyramide from molecular  
 probes T-20192

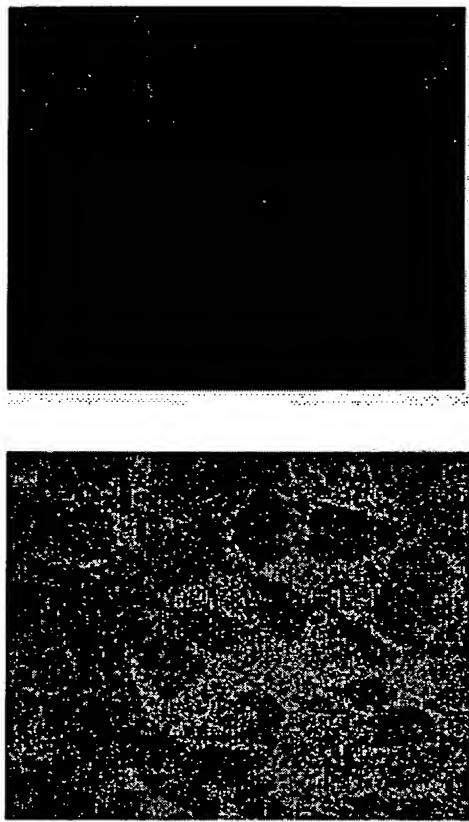
**FIG. 15A**

**Procedure for immunofluorescence (permeabilized cells)****FIG. 15B**

Immunofluorescence of surface exposed HSC70

MCF-7

Non-permeabilized



MCF-7/AR

Permeabilized

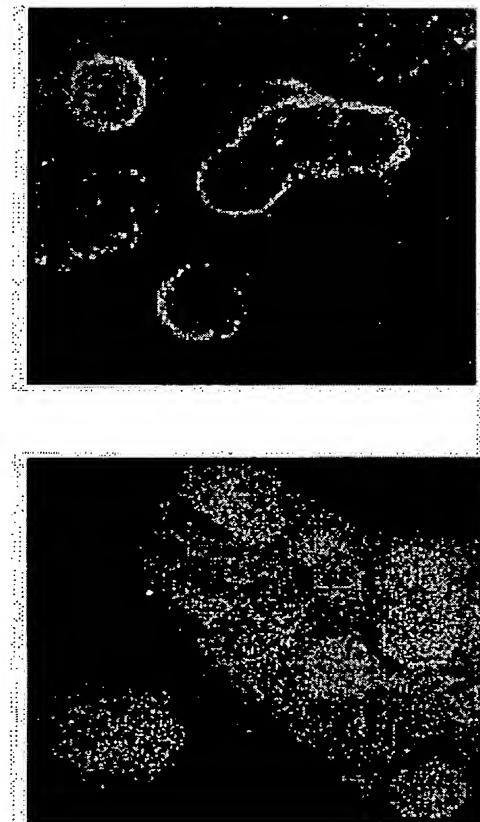
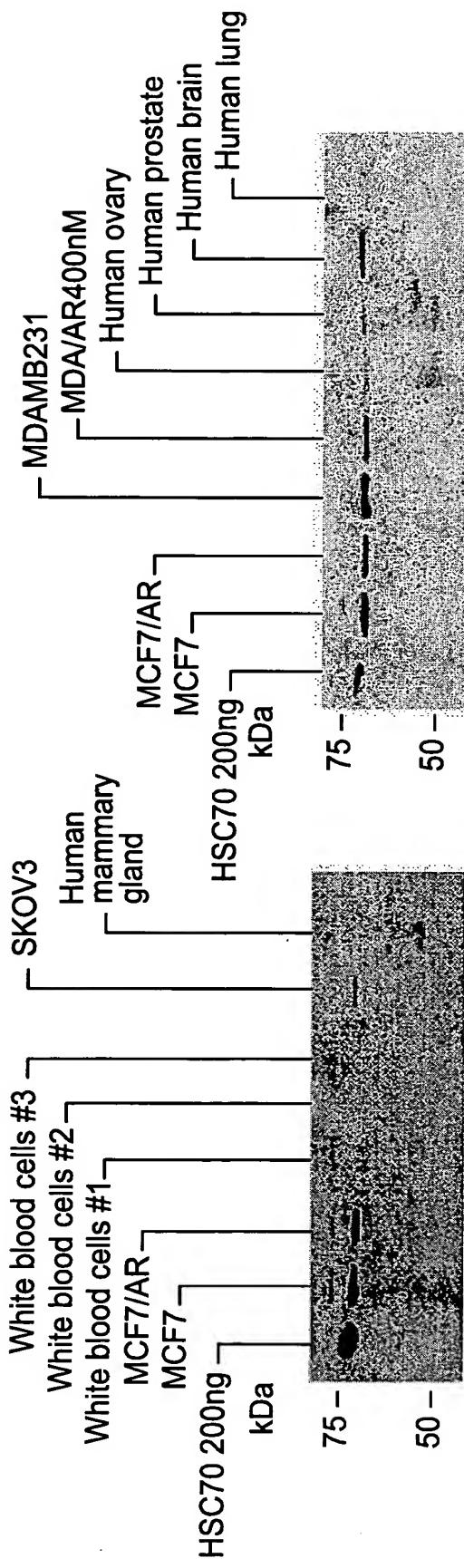


FIG. 16

**HSC70 expression****FIG. 17**

**FIG. 18**

